Biochem. J. (1995) 305, 843–851 (Printed in Great Britain)

Interleukin-3 facilitates glucose transport in a myeloid cell line by regulating the affinity of the glucose transporter for glucose: involvement of protein phosphorylation in transporter activation

Michael V. BERRIDGE* and An S. TAN
Malaghan Institute of Medical Research, Wellington School of Medicine, P.O. Box 7060, Wellington South, New Zealand

Growth factors promote cell survival and proliferation by activating signal transduction pathways that result in progression through the cell cycle and differential gene expression. Uptake of simple sugars needed for basal cell metabolism, and for macromolecular synthesis necessary for cell growth and proliferation, is thought to follow as a consequence of signal transduction to the nucleus. However, in the presence of inhibitors of DNA synthesis and respiration, growth factors can still promote cell survival responses in the short term, raising the possibility that they may also regulate critical membrane and cytosolic processes necessary for cell survival. We have tested this hypothesis directly by investigating the role of the haemopoietic growth factor, interleukin-3 (IL-3), in the regulation of glucose transport in the bone marrow-derived cell line, 32D. We show that IL-3 promotes glucose transport by actively maintaining the affinity of the plasma membrane glucose transporter for glucose (K_m 1.35 ± 0.15 mM, $n = 4$). Withdrawal of IL-3 for 1 h resulted in reduced affinity for glucose (K_m 2.96 ± 0.28 mM, $n = 4$) without an associated change in $V_{max.}$. Furthermore, glucose transporter molecules at the cell surface, as determined by cytochalasin B binding to isolated plasma membranes, did not differ significantly between control and IL-3-treated cells. Inhibition of DNA synthesis with mitomycin C or with the respiratory poison, sodium azide, did not affect the ability of IL-3 to promote glucose transport. In contrast, the tyrosine kinase inhibitors genistein and erbstatin extensively inhibited control and IL-3-stimulated glucose transport, some preference for IL-3-stimulated responses being observed at low inhibitor concentrations. The light-activated protein kinase C inhibitor, calphostin C, also inhibited control and IL-3-stimulated glucose transport but without preference for IL-3 responses. Additionally, the tyrosine phosphatase inhibitor, orthovanadate, stimulated control and IL-3-dependent glucose transport by 50–80% while the protein kinase A inhibitor, KT5720, inhibited glucose transport by about 20% at plateau values. These results indicate that IL-3 is involved in continuous maintenance of glucose transporter activity by a mechanism that involves tyrosine kinases and protein kinase C, and demonstrate that this activation is not dependent on respiration or signal transduction to the nucleus.

INTRODUCTION

Most mammalian cells are dependent on an external supply of glucose to maintain basal metabolism necessary for survival, and to provide for the metabolic demands of cell growth, proliferation and function. Transport of glucose across the plasma membrane is accomplished by a family of 'facilitative' glucose transporter molecules which shift glucose down its concentration gradient towards equilibrium [1]. In insulin-responsive tissues, glucose transport is regulated in the short term (min) by recruitment of stored transporter molecules, primarily GLUT-4, from an intracellular pool to the plasma membrane [2–5]. In addition, several studies have shown that insulin can reduce transporter K_m for glucose while increasing $V_{max.}$ [6,7]. These results could be explained by a two-receptor translocation model [8], or by intrinsic activation of glucose transporter molecules [1,9]. With murine adipocytes [10] and human fibroblasts [11], insulin and other growth factors failed to phosphorylate glucose transporters, while other agents that increased GLUT-4 phosphorylation, including high intracellular calcium [12,13], isoprenaline (isoproterenol) [14], okadaic acid [15,16] and parathyroid hormone [17], inhibited insulin-dependent glucose uptake. These results suggest that insulin may regulate glucose transport by activating protein phosphatase enzymes, thus converting GLUT-4 into an unphosphorylated state. In support of this model, Begum and Draznin [18] have recently shown that in diabetic rats, insulin failed to promote GLUT-4 dephosphorylation and recruitment to the plasma membrane. To a lesser extent, the universal glucose transporter, GLUT-1, is also recruited to the cell surface of adipocytes in response to insulin [19,20]; however, whether this recruitment is associated with increased intrinsic activity of GLUT-1 is not known. Evidence for suppressed intrinsic activity of GLUT-1 following induced differentiation of 3T3-L1 fibroblasts to adipocytes has been presented [21], and the action of insulin on undifferentiated 3T3-L1 fibroblasts appears to involve a significant increase in the intrinsic activity of GLUT-1 [22].

With non-insulin-dependent cells, intrinsic activation of glucose transporter molecules has been demonstrated by Nefesh et al. [23] who used the bone marrow-derived lymphoid cell line, Ba/F3, to show that within 30 min, interleukin 3 (IL-3) and insulin-like growth factor 1 (IGF-1) treatment decreased transporter K_m for glucose while leaving $V_{max.}$ unchanged. In this

Abbreviations used: CSF-1, colony-stimulating factor 1; 2DOG, 2-deoxy-D-glucose; G-CSF, granulocyte colony-stimulating factor; GF, glucose-free; IGF-1, insulin-like growth factor 1; IL-2, interleukin 2; IL-3, interleukin 3; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PKC, protein kinase C; SF, serum-free.

* To whom correspondence should be addressed.

system, GLUT-1 is probably the major glucose transporter subtype, as suggested by its expression on cultured haemopoietic cells [24]. Other evidence that rapid increases in glucose transport are associated with the action of haemopoietic growth factors has been presented by Whetton et al. for IL-3 [25] and Hamilton et al. for colony-stimulating factor 1 (CSF-1) [26], but neither of these studies investigated kinetic parameters associated with increased glucose uptake.

Increased affinity of glucose transporters for glucose has also been demonstrated in tumorigenic revertants of suppressed hybrid cells [27], and this may explain the increased glycolysis known to be associated with tumour cells [28].

In the present study we have used the bone marrow-derived cell line, 32D, to show that IL-3 actively promotes hexose transport by maintaining the affinity of the glucose transporter for glucose without affecting either $V_{max.}$ or the level of glucose transporter molecules in the plasma membrane. Both control and IL-3-stimulated glucose transport were completely inhibited by the tyrosine kinase inhibitors, genistein and erbstatin, and by the protein kinase C (PKC) inhibitor, calphostin C, but were only marginally sensitive to the protein kinase A inhibitor, KT5720. In contrast, the tyrosine phosphatase inhibitor, orthovanadate, stimulated glucose transport in the presence or absence of IL-3. These results demonstrate that both tyrosine kinase and PKC are required for the maintenance of glucose transport, and suggest that IL-3-dependent intrinsic activation of the glucose transporter is involved.

MATERIALS AND METHODS

Chemicals

D-Mannitol, D-glucose, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sodium azide, sodium orthovanadate and cytochalasin B were from Sigma Chemical Co. (St. Louis, MO, U.S.A.), 2-deoxy-D-glucose (2DOG) from Fluka (Buchs, Switzerland), genistein from Gibco-BRL, calphostin C and KT5720 from Kamiya Biomedical Co. (Thousand Oaks, CA, U.S.A.), and mitomycin C from Kyowa Hakko Kogyo Co. (Tokyo, Japan). Erbstatin was obtained from Professor Bruce Baguley, Cancer Research Laboratories, Auckland School of Medicine, New Zealand.

Cells and cell culture

32D clone 23 cells (32D), originally derived from long-term bone marrow cultures of C3H/HeJ mice [29], were obtained from Professor J. D. Watson (University of Auckland, School of Medicine, Auckland, New Zealand). Cells were maintained in RPMI 1640 medium (Gibco-BRL, Grand Island, NY, U.S.A.) supplemented with 25 μg/ml penicillin, 25 μg/ml streptomycin, 10% (v/v) fetal calf serum and 7.5% WEHI-3-conditioned medium as a source of IL-3, or supplied with pure recombinant murine IL-3 obtained from Professor Watson (sourced from Immunex, Seattle, WA, U.S.A.). Cells were cultured at 37 °C in a humidified incubator maintained at 5% CO_2. 32D.IL2, 32D.Epo and 32D.G were derived from 32D as described previously [30] and were maintained on their respective recombinant growth factors.

Glucose uptake

Glucose uptake was determined by the zero trans method in which cells (4×10^5/ml) were cultured in serum-free (SF)-RPMI 1640 containing 2 ng/ml IL-3 for 16 h. Under these conditions cell viability was maintained but cell proliferation ceased. Cells were then washed three times with SF-RPMI 1640, resuspended at 5×10^6 cells/ml and treated with or without growth factor and/or inhibitors in a total volume of 0.5 ml for 60 min with intermittent mixing. Cells were then collected by centrifugation, resuspended in 250 μl of glucose-free (GF)-RPMI 1640 and preincubated for 3 min at 37 °C. [^{3}H]2-deoxy-D-glucose (1 μCi; Amersham, U.K.) was then added at a final 2DOG concentration of 100 μM. Glucose uptake was determined at 37 °C over 45 s under conditions where glucose uptake was linear for at least 3 min. Uptake was stopped by adding 250 μl of ice-cold GF-RPMI 1640 containing 0.3 mM phloretin, chilling on ice, and centrifuging through a cushion of ice-cold 10% (w/v) BSA (50 μl) at 8800 g for 30 s in a microcentrifuge. The supernatant was discarded and the tube rinsed with 250 μl of GF-RPMI. The pellet was lysed in 100 μl of 1% Triton X-100 and radioactivity determined in a liquid-scintillation spectrophotometer. Results are presented as the average of duplicate determinations ± S.E.M.

Isolation of cell membranes

Cell membranes were isolated from 32D cells by one of two methods.

Method A

Crude cell membranes were prepared from 32D cells by hypotonic lysis according to the method of Esko et al. [31]. Briefly, 32D cells suspended in PBS were added dropwise to 1 mM Tris/HCl, pH 7.2, such that the final cell concentration was 5×10^6 cells/ml and the PBS:Tris/HCl ratio 1:3. These hypotonically lysed cells were centrifuged at 3000 g for 15 min and the pellet resuspended in 1 mM Tris/HCl, pH 7.2. Nuclei were separated from membranes by pipetting and centrifugation at 3000 g for 10 min. Cell membranes in the supernatant were recovered by centrifugation at 48500 g for 1 h. The membrane pellet was resuspended in 1 mM Tris/HCl, pH 7.2, and stored at −20 °C.

Method B

Plasma membranes were isolated from 32D cells following the *N*-hydroxysuccinimide–biotin cell-labelling method of Shetty et al. [32]. Cells were washed with PBS and resuspended in ice-cold bicarbonate buffer (120 mM NaCl, 30 mM $NaHCO_3$, 5 mM KCl, pH 8.5). An equal volume of 0.2 mg/ml sulphosuccinimidyl-6-(biotinamido)hexanoate (Pierce, Rockford, IL, U.S.A.) in bicarbonate buffer was added and the cells left at 4 °C for 1 h with intermittent resuspension. Cells were washed three times in washing buffer (140 mM NaCl, 20 mM Tris/HCl, 5 mM KCl, pH 7.5) and the pellet resuspended in 1 ml of hypotonic homogenization buffer containing 10 mM $NaHCO_3$, 10 μM dimethyl sulphoxide, 2 μg/ml aprotinin and 1 mM EDTA. Cells were allowed to swell on ice for 10 min after which they were disrupted by 50 strokes of a motor-driven homogenizer with a Teflon pestle. Osmolarity was restored by adding 0.1 ml of 1.5 M NaCl/100 mM Tris/HCl (pH 7.0) and the homogenate centrifuged at 12700 g for 15 s in a microfuge to sediment nuclei. The postnuclear supernatant was added to 0.5 ml of streptavidin–agarose beads (Gibco/BRL, Grand Island, NY, U.S.A.) pre-equilibrated with homogenization buffer and protease inhibitors. After adsorption at 4 °C for 30 min with occasional mixing, beads were recovered and washed three times with homogenization buffer. The final pellet containing the plasma membrane which represents 3% of the total membrane protein was resuspended in 1 ml of buffer and stored at −20 °C, together with the plasma membrane-depleted fraction, for protein determination and cytochalasin B binding.

[^{3}H]Cytochalasin B binding

Glucose-inhibitable [^{3}H]cytochalasin B binding to membranes was determined, in the presence and absence of 200 mM D-glucose or mannitol (essentially as described by Salter and Weber [33]), by incubating membrane preparations in a total volume of 0.8 ml with 0.4 μCi (16.6 Ci/mmol) [^{3}H]cytochalasin B (Amersham, U.K.) for 20 min at 20 °C. Membranes were recovered at 210000 *g* for 1 h in a Beckman 70.1Ti rotor. The membrane pellet and tube were rinsed carefully with 0.1 ml of PBS, care being taken not to disturb the pellet. Membranes were resuspended in 100 μl of 1% Triton X-100 prior to determining radioactivity in a liquid-scintillation counter.

Cellular MTT assay

The MTT assay for cell proliferation was a modification [34] of the original method of Mosmann [35]. Briefly, 2×10^4 cells in 0.1 ml of culture medium were incubated in microtitre plates (Nunc, Roskilde, Denmark) for periods up to 24 h before adding 15 μl of 5 mg/ml MTT. After 2 h at 37 °C, 100 μl of lysing buffer (10% SDS, 45% dimethylformamide adjusted to pH 4.7 with acetic acid) was added and mixed to dissolve the blue formazan crystals, and the absorbance determined at 570 nm in a Titretek Multiskan microtitre plate reader.

The MTT assay can be considered to be a sensitive measure of glucose uptake and utilization by the cell. Contrary to the commonly held view, we have recently shown that most cellular MTT reduction is not associated with mitochondria but involves NADH- and NADPH-dependent mechanisms that are insensitive to respiratory chain inhibitors [36].

[^{3}H]Thymidine incorporation assay

DNA synthesis was measured by incubating 2×10^4 cells in 0.1 ml of culture medium in microtitre plates for periods up to 24 h before adding 0.5 μCi of [^{3}H]thymidine (Amersham, U.K.) for 3 h. Incorporation of radioactivity into DNA was determined using an automated cell harvester and liquid-scintillation counter.

Protein determination

Protein was determined using a microplate adaptation of the Lowry method [37] and the absorbance was measured at 570 nm. Serial dilutions of cell fractions were analysed in the linear range using BSA as a standard.

Cell viability assay

Cell viability was determined by Trypan Blue exclusion using a haemocytometer.

RESULTS

Effects of IL-3 on 2DOG uptake by 32D cells

Survival and proliferation of the bone marrow-derived cell line, 32D, is dependent on IL-3. Withdrawal of IL-3 results in rapid loss of viable cells and loss of ability to reduce the tetrazolium salt, MTT [30], a sensitive indicator of glucose uptake and energy utilization [36]. To investigate directly the effects of IL-3 on glucose uptake, 32D cells were cultured overnight with limiting concentrations of IL-3 (2 ng/ml) in the absence of serum. Cells were then treated in the presence or absence of IL-3 (20 ng/ml) and [^{3}H]2DOG uptake determined. Table 1(A) shows that IL-3 stimulated 2DOG uptake within 30 min of IL-3 treatment and that uptake reached a maximum by 60–120 min. More detailed analysis of 2DOG uptake over the initial 1 h following IL-3

Table 1 Effect of IL-3 on 2DOG uptake by 32D cells

32D cells were deprived of serum in the presence of 2 ng/ml IL-3 for 16 h then treated as follows: (A) with or without 20 ng/ml IL-3 for the times indicated and (B) with increasing concentrations of IL-3 for 60 min. The initial rate of [^{3}H]2DOG (1 μCi, 100 μM) uptake was then measured over 45 s. Results are the average of duplicates ± S.E.M.

		2DOG uptake	
Cell treatment	Time (min)	(c.p.m.) ± S.E.M.	(% of control)
(A) Time course			
Control	30	2505 ± 26	100 ± 1
IL-3	30	3507 ± 100	140 ± 4
Control	60	3215 ± 414	100 ± 12
IL-3	60	5435 ± 164	169 ± 5
Control	120	2133 ± 47	100 ± 2
IL-3	120	4507 ± 89	211 ± 4
Control	240	2971 ± 183	100 ± 6
IL-3	240	4487 ± 211	151 ± 7
(B) Concentration course			
0 ng/ml IL-3	60	6113 ± 134	100 ± 3
2 ng/ml IL-3	60	9230 ± 226	151 ± 4
20 ng/ml IL-3	60	10378 ± 194	170 ± 3
100 ng/ml IL-3	60	11206 ± 227	183 ± 4
200 ng/ml IL-3	60	13070 ± 321	191 ± 2
300 ng/ml IL-3	60	11877 ± 98	194 ± 2

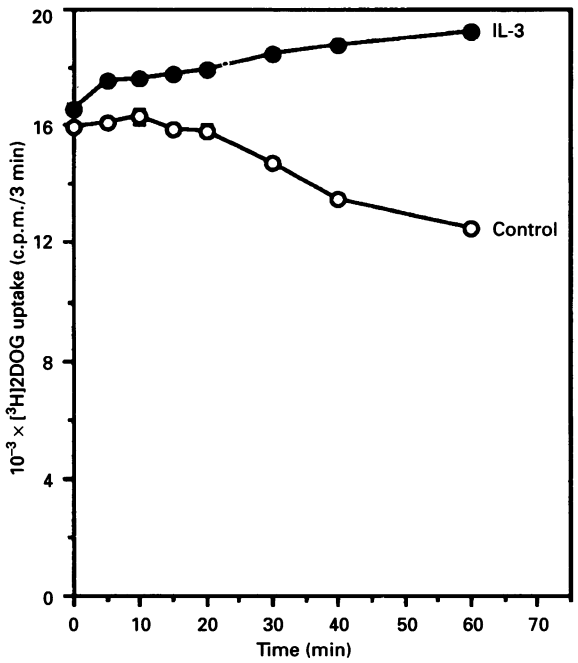

Figure 1 Early effects of IL-3 on 2DOG uptake by 32D cells

Serum-starved cells maintained on 2 ng/ml IL-3 for 16 h were treated with (●) or without (○) 20 ng/ml IL-3 for the times indicated and the initial rate of [^{3}H]2DOG (1 μCi, 100 μM) uptake measured over 3 min. Results are the average of duplicate determinations ± S.E.M.

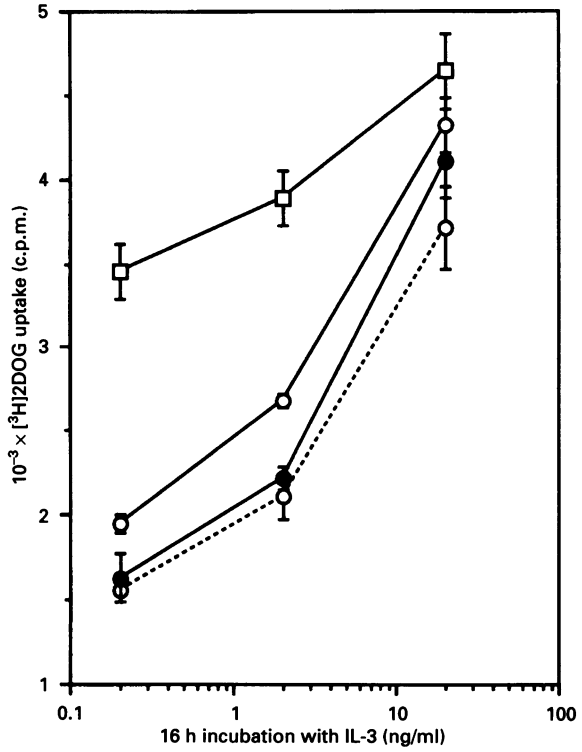

Figure 2 Effect of IL-3 pretreatment on IL-3 stimulation of 2DOG uptake

32D cells were treated with increasing concentrations of IL-3 for 16 h in the absence of serum prior to determining the effect of IL-3 treatment (broken line, control; ●, 0.2 ng/ml IL-3; ○, 2 ng/ml IL-3; □, 20 ng/ml IL-3) for 1 h on 2DOG uptake. Results are the average of duplicate determinations ± S.E.M.

Table 2 Effect of haemopoietic growth factors on 2DOG uptake by 32D cells and derived cell lines

Cells grown on optimum concentrations of their respective recombinant growth factors were deprived of serum in the presence of 2 ng/ml IL-2, IL-3 or G-CSF, or 0.2 unit/ml erythropoietin for 16 h. Cells were then treated with or without growth factor for 1 h after which the initial rate of [^{3}H]2DOG (1 μCi, 100 μM) uptake was measured over 45 s. Results are the average of duplicates ± S.E.M. Abbreviation: CytB, cytochalasin B.

Cell line	Treatment (1 h)	2DOG uptake (nmol/10^6 cells per min)	2DOG uptake (% of control)
32D	–	0.101 ± 0.006	100 ± 6
	IL-3 (20 ng/ml)	0.181 ± 0.016	179 ± 16
	IL-3 + CytB (10 μg/ml)	0.018 ± 0.004	−18 ± 4
32DEpo	–	0.080 ± 0.004	100 ± 5
	Epo (2 units/ml)	0.158 ± 0.008	198 ± 10
32DIL-2	–	0.101 ± 0.014	100 ± 14
	IL-2 (20 ng/ml)	0.173 ± 0.007	171 ± 7
32DG	–	0.047 ± 0.004	100 ± 8
	G-CSF (20 ng/ml)	0.082 ± 0.004	174 ± 8

treatment and withdrawal used 3 min 2DOG uptake times instead of 45 s to reduce standard errors. The results show that the difference in 2DOG uptake between control and IL-3-treated cells is contributed to by a real stimulation following the shift from 2 to 20 ng/ml IL-3, and by a decline in 2DOG uptake following IL-3 withdrawal (Figure 1). Furthermore, significant

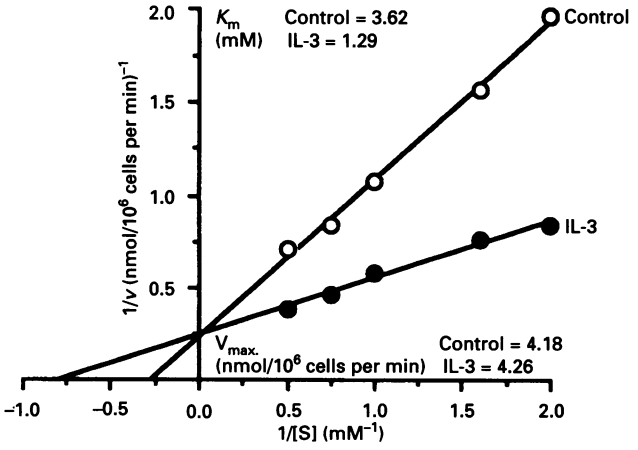

Figure 3 Lineweaver–Burk analysis of 2DOG uptake by 32D cells

Serum-starved cells maintained on 2 ng/ml IL-3 for 16 h were treated with (●) or without (○) 20 ng/ml IL-3 for 1 h prior to determining 2DOG (1 μCi) uptake over a range (0.2–2.0 mM) of 2DOG concentrations.

Table 3 Effect of IL-3 treatment on [^{3}H]cytochalasin B binding to plasma membranes from 32D cells

32D cells were deprived of serum in the presence of 2 ng/ml IL-3 for 16 h after which they were treated with or without 20 ng/ml IL-3 for 1 h. Cells were then harvested by centrifugation. In experiment A plasma membranes or, in experiment B unfractionated cell membranes, were prepared as described in the Materials and methods section. Glucose-inhibitable [^{3}H]cytochalasin B (CytB) binding to membranes was then determined. Results are the average of duplicate determinations ± S.E.M. Specific cytochalasin B binding was derived by subtracting D-glucose competition from the mannitol control binding.

Cell fraction	Assay conditions	Total [^{3}H]CytB binding (c.p.m.)	Specific CytB binding (c.p.m./assay)	Specific CytB binding (pmol/mg of protein)
(A) Plasma membranes				
Control	–	6185 ± 685		
	Mannitol (200 mM)	5432 ± 265		
	D-Glucose (200 mM)	3161 ± 306		
			2271 ± 286	19.0 ± 2.4
IL-3	–	5681 ± 126		
	Mannitol (200 mM)	6436 ± 86		
	D-Glucose (200 mM)	4095 ± 510		
			2341 ± 366	21.0 ± 3.3
Plasma membrane-depleted fraction				
Control	–	6951 ± 403		
	Mannitol (200 mM)	7544 ± 342		
	D-Glucose (200 mM)	4100 ± 475		
			3444 ± 414	2.4 ± 0.3
IL-3	–	5845 ± 517		
	Mannitol (200 mM)	7933 ± 245		
	D-Glucose (200 mM)	3972 ± 305		
			3961 ± 290	3.0 ± 2.2
(B) Unfractionated cell membranes				
Control	–	7583 ± 135		
	Mannitol (200 mM)	7445 ± 441		
	D-Glucose (200 mM)	4201 ± 67		
			3382 ± 202	8.3 ± 0.5
IL-3	–	7737 ± 273		
	Mannitol (200 mM)	7647 ± 625		
	D-Glucose (200 mM)	4239 ± 456		
			3498 ± 729	10.5 ± 2.2

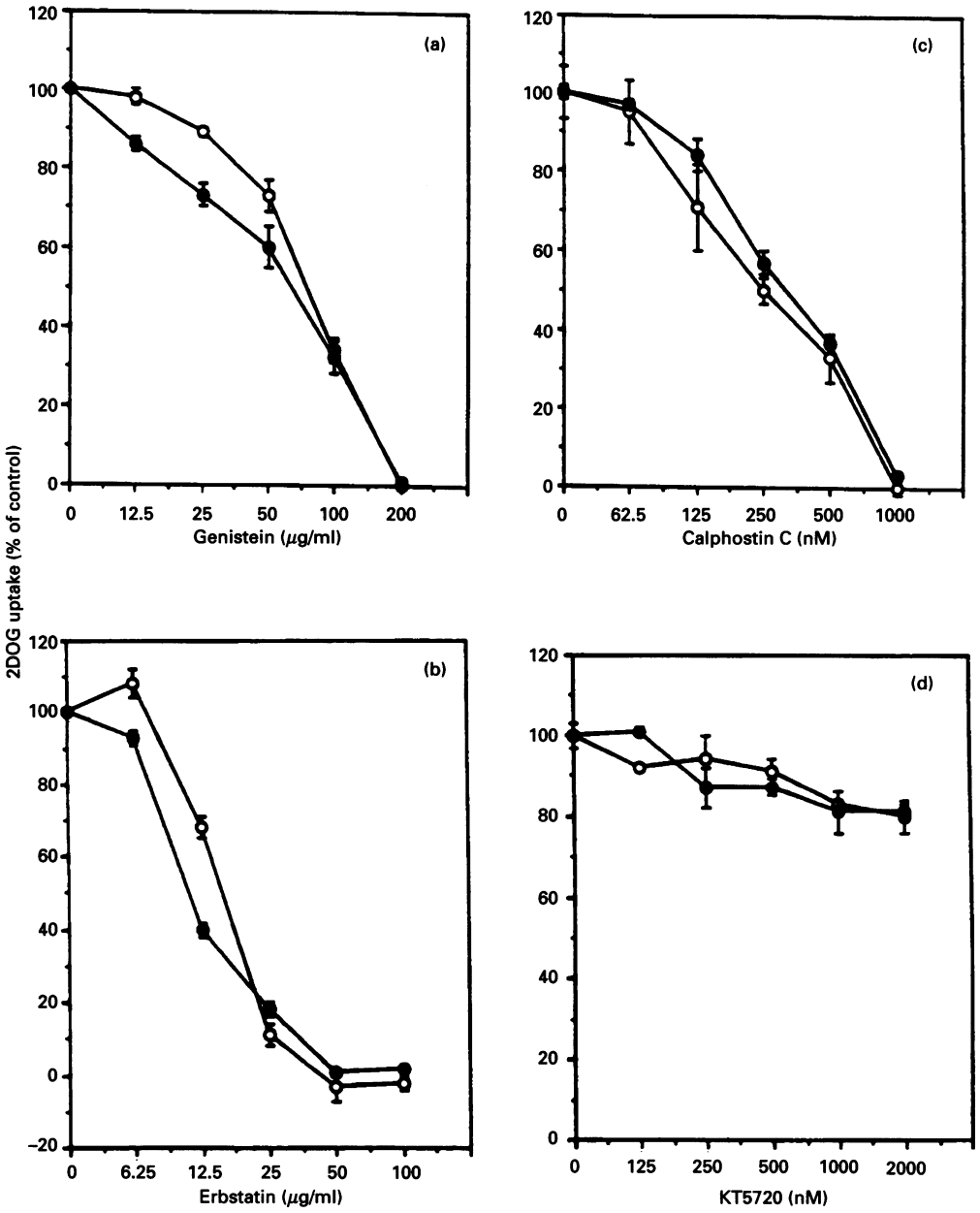

Figure 4 Effect of protein kinase inhibitors on control and IL-3-stimulated 2DOG uptake

Serum-starved cells maintained on 2 ng/ml IL-3 for 16 h were treated with (●) or without (○) 20 ng/ml IL-3 for 1 h in the presence of increasing concentrations of (**a**) genistein, (**b**) erbstatin, (**c**) calphostin C and (**d**) KT5720 before determining 2DOG uptake. Results are the average of duplicate determinations ± S.E.M.

differences in glucose uptake between control and IL-3-treated cells were observed at the earliest time point (i.e. the 3 min uptake period at time zero). Increased 2DOG uptake was dependent on the concentration of IL-3 used, plateau response being observed at 200–300 ng/ml IL-3 (Table 1B). Because 70–80% of the maximum response was evident at 20 ng/ml IL-3, this concentration was used routinely in subsequent experiments.

The ability of IL-3 to stimulate 2DOG uptake was dependent on the past history of the cells. Initial experiments carried out without overnight serum starvation were characterized by high backgrounds and reduced IL-3 signal. Culturing cells overnight with 20 ng/ml IL-3 also resulted in reduced signal to noise ratios (Figure 2), while the use of 0.2 ng/ml resulted in an acceptable IL-3 response, but some loss of cell viability was observed (67% viable cells compared with 94–95% viability with 2–20 ng/ml IL-3). Thus, overnight culture with 2 ng/ml IL-3 in the absence of serum followed by treatment for 1 h with or without 20 ng/ml IL-3 was chosen for optimal 2DOG response to IL-3.

Effects of erythropoietin, interleukin 2 (IL-2) and granulocyte colony-stimulating factor (G-CSF) on 2DOG uptake by 32D cells

That enhanced glucose uptake is not restricted to IL-3 is shown

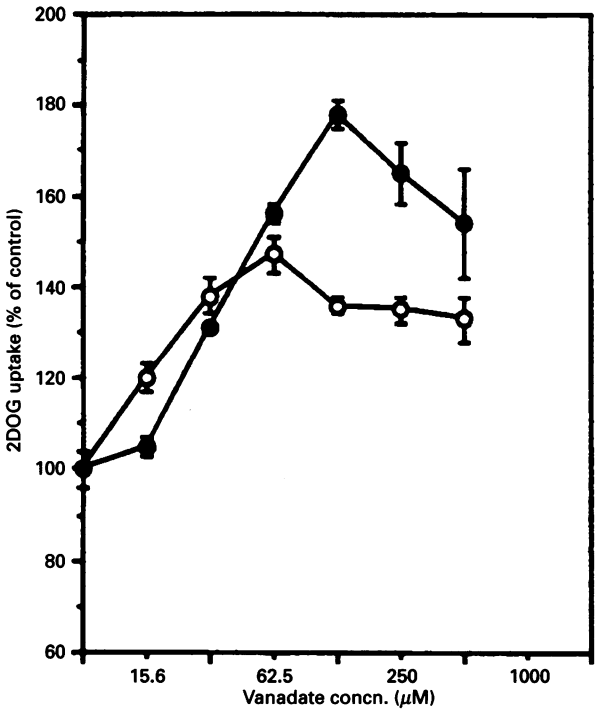

Figure 5 Effect of orthovanadate on control and IL-3-stimulated 2DOG uptake

Serum-starved 32D cells maintained on 2 ng/ml IL-3 for 16 h were treated with (●) or without (○) IL-3 in the presence of increasing concentrations of sodium orthovanadate. Results are the average of duplicate determinations ± S.E.M.

in Table 2. Using 32D cell lines selected for growth on erythropoietin, IL-2 and G-CSF, increased 2DOG uptake was also observed with each of these haemopoietic growth factors. Table 2 also shows that increased glucose uptake in response to growth factor was sensitive to cytochalasin B, a potent inhibitor of facilitative glucose transport [38].

Effect of IL-3 on the intrinsic activity of the glucose transporter on 32D cells

Increased glucose uptake in response to IL-3 could result from increased transporter expression on the cell surface, or from a change in the intrinsic properties of the transporter. To determine the effect of IL-3 on the kinetic properties of the glucose transporter, serum-starved 32D cells were treated with or without IL-3 for 1 h and 2DOG uptake determined over a range of 2DOG concentrations. Figure 3 shows Lineweaver–Burk analysis of the results from a representative experiment. Over several experiments ($n = 4$), a 2–3-fold difference in transporter K_m for glucose was observed between control and IL-3-treated cells (K_m for control 2.96 ± 0.28 mM; K_m for IL-3 1.35 ± 0.15 mM) while $V_{max.}$ remained unchanged (control, 3.42 ± 0.53 nmol/10^6 cells per min; IL-3, 3.15 ± 0.34 nmol/10^6 cells per min). The difference in K_m for glucose between control and IL-3-treated cells was shown to result from reduced transporter affinity for glucose in cells deprived of IL-3. Thus 32D cells growing exponentially in WEH1-3-conditioned medium exhibited a K_m for glucose of 1.24 ± 0.07 mM ($n = 8$) while cells deprived of serum but maintained in 2 ng/ml IL-3 for 16 h had a K_m value of 1.48 ± 0.14 mM ($n = 3$).

Effect of IL-3 on glucose transporter expression on 32D cells

The possibility that increased glucose transport following IL-3 treatment (Figure 1) may be contributed to by increased expression of glucose transporter molecules on the cell surface was investigated by determining [^{3}H]cytochalasin B binding to plasma membranes from 32D cells. Table 3 shows that there were no significant differences in glucose-inhibitable [^{3}H]cytochalasin B binding to purified plasma membranes or crude cell membranes between control and IL-3-treated cells.

Involvement of tyrosine kinase in IL-3-stimulated glucose transport

The mechanism by which IL-3 stimulates glucose transport in 32D cells was investigated with protein kinase inhibitors (Figure 4). The tyrosine kinase inhibitor, genistein, showed a reproducible ability to preferentially inhibit IL-3-stimulated glucose transport at low genistein concentrations (10–50 μg/ml) when compared with control cells deprived of IL-3 (Figure 4a). This pattern of inhibition was not evident at inhibitor concentrations above 50 μg/ml where both control and IL-3-stimulated glucose transport were equally affected by genistein. Complete inhibition of glucose transport was observed at 200 μg/ml genistein. Similar results were obtained with an alternative tyrosine kinase inhibitor, erbstatin (Figure 4b).

A different pattern of inhibition was observed with the highly specific, light-activated PKC inhibitor, calphostin C. Thus, in two separate experiments, glucose uptake in control cells was marginally more sensitive to calphostin C throughout most of the inhibitor concentration range than was uptake into IL-3-treated cells (Figure 4c). Complete inhibition of glucose transport was achieved at 1 μM calphostin C, whether or not IL-3 was present.

In contrast with genistein, erbstatin and calphostin C, which strongly inhibited glucose uptake, the protein kinase A inhibitor, KT5720, resulted in less than 20% reduction of glucose transport at plateau values, and inhibition curves did not differ significantly between IL-3-treated and control cells (Figure 4d).

Effect of orthovanadate on control and IL-3-stimulated 2DOG uptake

The tyrosine phosphatase inhibitor, orthovanadate, was also used to investigate the role of phosphorylation in control and IL-3-stimulated glucose transport. Vanadate caused a concentration-dependent increase in glucose transport both in the presence and absence of IL-3 (Figure 5).

Effects of metabolic inhibitors on control and IL-3-stimulated 2DOG uptake

It is possible that inhibition of control and IL-3-stimulated glucose transport by tyrosine kinase and PKC inhibitors may result from prior effects of these inhibitors on DNA synthesis and gene expression rather than an immediate effect on glucose transport. To test this possibility, inhibitors of DNA synthesis (mitomycin C, 10 μg/ml) and respiration (sodium azide, 2 mM) that are known to extensively inhibit [^{3}H]thymidine incorporation into DNA at early times (see [39] and Figure 6) were used. Table

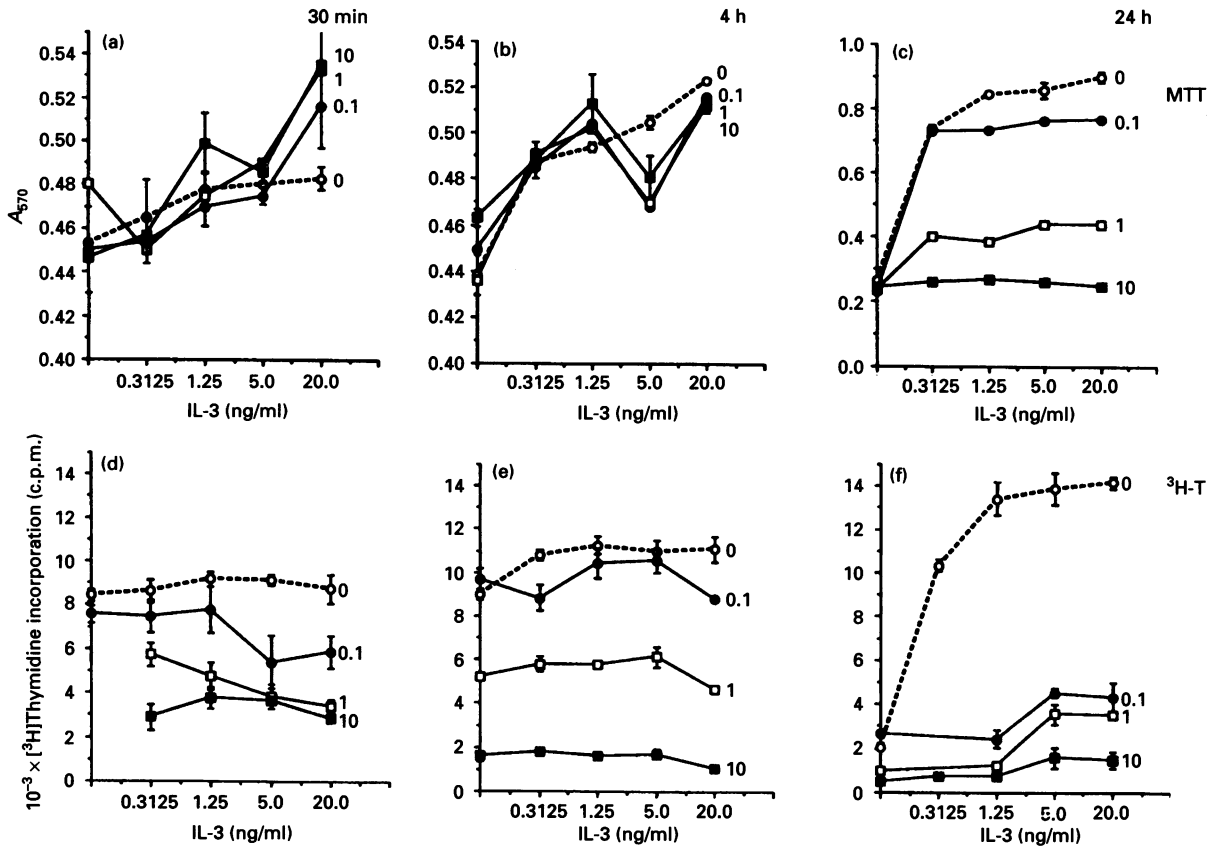

Figure 6 Effect of mitomycin C on MTT responsiveness and [^{3}H]thymidine (^{3}H-T) incorporation into 32D cells treated with increasing concentrations of IL-3

Cells were cultured for 30 min (**a** and **d**), 4 h (**b** and **e**) or 24 h (**c** and **f**) without (○) or with mitomycin C at 0.1 μg/ml (●), 1 μg/ml (□), or 10 μg/ml (■) prior to determining MTT reduction (**a–c**) and [^{3}H]thymidine incorporation (**d–f**). Results are the average of duplicate determinations ± S.E.M.

Table 4 Effect of metabolic inhibitors on control and IL-3-stimulated 2DOG uptake

32D cells were deprived of serum in the presence of 2 ng/ml IL-3 for 16 h then treated with or without 20 ng/ml IL-3 for 1 h in the presence or absence of mitomycin C, sodium azide or cytochalasin B. The initial rate of [^{3}H]2DOG (1 μCi, 100 μM) uptake was then measured over 45 s. Results are the average of duplicates of ± S.E.M.

		2DOG uptake	
Inhibitor	IL-3	(c.p.m. ± S.E.M.)	(% of control)
None	−	3215 ± 32	100 ± 1
	+	5851 ± 64	182 ± 2
Mitomycin C (10 μg/ml)	−	3005 ± 52	99 ± 2
	+	6141 ± 48	191 ± 1
Sodium azide (2 mM)	−	3183 ± 24	99 ± 7
	+	5337 ± 72	166 ± 2
Cytochalasin B (10 μg/ml)	−	65 ± 95	2 ± 3
	+	16 ± 38	0 ± 2

4 shows that neither background nor IL-3-stimulated glucose uptake were affected by these inhibitors under conditions where glucose uptake was completely inhibited by cytochalasin B (10 μg/ml). Thus both glucose uptake and MTT reduction appear to be regulated by IL-3, by a mechanism that is not dependent on DNA synthesis, gene expression and respiration.

DISCUSSION

In this study we show that glucose uptake by a myeloid cell line is regulated by IL-3 independently of DNA synthesis and cell proliferation, via a mechanism that involves both tyrosine kinases and PKC. Kinetic analysis of glucose transport following IL-3 withdrawal showed that IL-3 acts by maintaining the affinity of the plasma membrane glucose transporter for glucose. Withdrawal of IL-3 resulted in a 2–3-fold increase in transporter K_m for glucose within 1 h. Loss of affinity for glucose was not associated with a change in $V_{max.}$ and no decline in glucose transporter molecules on plasma membranes was evident. Furthermore, the changes in glucose transport observed were not associated with changes in the cell cycle status of the cells (M. V. Berridge and A. S. Tan, unpublished work) which has been shown to influence glucose uptake in a cycling lymphoid cell line [40]. These results verify and extend observations made by Nefesh et al. [23] who used the lymphoid cell line, Ba/F3, to show that IL-3 facilitated 2DOG uptake within 15 min of IL-3 treatment, and by 30 min caused a 1.6–2.6-fold decrease in transporter K_m for glucose when compared with untreated control cells deprived of IL-3. Our results show that the observed changes in glucose transport are not associated with increased glucose transporter expression on the cell surface, and dem-

onstrate that the continued presence of IL-3 is necessary to maintain the K_m of the glucose transporter within the range of 1.0–1.5 mM ($n = 15$). This interpretation is supported by inhibitor studies which show rapid loss of glucose transporter function in the presence of the tyrosine kinase inhibitors genistein and erbstatin, and the PKC inhibitor calphostin C. In contrast, the metabolic inhibitors mitomycin C and sodium azide failed to affect glucose transport over a 1 h period at which time DNA synthesis was markedly inhibited. These inhibitors also failed to affect cellular reduction of the tetrazolium salt, MTT, adding support to the view that MTT reduction is more closely related to glucose uptake than to respiration and nucleic acid synthesis ([37], Figure 6).

Inhibition of glucose transport by the PKC inhibitor, calphostin C, and to a much lesser extent by the protein kinase A inhibitor, KT5720, (Figure 4) is in agreement with current knowledge concerning phosphorylation of glucose transporters. Thus the human erythrocyte glucose transporter, GLUT-1, is known to be a substrate for PKC both *in vitro* [41] and *in vivo* in response to phorbol esters [10]. In contrast, GLUT-4 is phosphorylated *in vitro* by protein kinase A on serine-488 [42] which is conserved between species, and site-directed mutagenesis studies have confirmed that this amino acid is phosphorylated in Chinese hamster ovary cells transfected with GLUT-4 [43].

Shifting cells from maintenance levels of IL-3 (2 ng/ml) to 20 ng/ml for 1 h stimulated 2DOG uptake by 20–40 %, whereas glucose uptake declined by a similar amount following IL-3 withdrawal (Figure 1). Taken together, these two effects account for the 50–80 % difference in glucose uptake observed between control cells deprived of IL-3 and cells treated with 20 ng/ml IL-3 for 1 h. Although neither $V_{max.}$ nor glucose transporters at the plasma membrane differed significantly between control and IL-3-treated cells (Figure 3, Table 3), a small 10–15 % increase in $V_{max.}$ that was not statistically significant resulted following the shift from 2 to 20 ng/ml IL-3. When the 10–20 % standard errors associated with these determinations are taken into account, a contributing effect of $V_{max.}$ on increased glucose uptake could have occurred. Furthermore, we did not determine glucose transporters on serum-deprived cells maintained in 2 ng/ml IL-3, raising the possibility of a small increase in plasma membrane glucose transporters following the shift to high IL-3. In contrast, the decline in 2DOG uptake following IL-3 withdrawal is readily explained by the increased K_m for glucose.

The view that cell growth factors may regulate glucose transport through associated signal transduction pathways, and that this activation may be mandatory for maintaining cell survival and for subsequent cell proliferation provides a conceptual shift in our understanding of cell growth regulation. Thus growth factor signalling may involve cellular targets other than nuclear events involved in DNA synthesis, mitosis and gene expression. Interestingly, the signalling events that regulate glucose transport include tyrosine kinases and PKC, both of which are also required for cell proliferation. However, neither DNA synthesis nor respiration appear to be required for IL-3 stimulation of glucose transport (Table 4), demonstrating that the regulation of glucose transport is not mediated through these processes, at least in the short term. Thus, it appears that growth factor signal transduction may include events involved in the regulation of glucose transport.

Although the molecular subtype of the glucose transporter on 32D cells has not been determined, it is likely that the erythrocyte/brain glucose transporter, GLUT-1, is predominant. Thus the affinity of the transporter for glucose on 32D cells (K_m 1.0–1.5 mM) is in general consistent with the known K_m for GLUT-1 on other proliferating haemopoietic cells and cultured cells [23,27,44], whereas GLUT-2 is known to exhibit a much lower affinity for glucose (see [38]). GLUT-4 is expressed primarily in insulin-responsive tissues [45] while GLUT-3 shows restricted expression in human tissues [46], and in rodents such as rats and mice is limited to the brain [47]. Although it is possible that other as yet uncharacterized glucose transporter subtypes occur on haemopoietic cells, most molecular and biochemical evidence supports a predominant role for GLUT-1 as the major transporter responsible for basal glucose transport, and its presence on most cells in culture.

Regulation of glucose transport in non-insulin-dependent cells is poorly understood. Increased glucose transport in response to insulin, IGF-1, IL-3 and CSF-1 has been demonstrated [23,25,26], and with 3T3 cells increased hexose transport in response to interleukin 1 has been shown to result from increased expression of glucose transporters [44]. With IL-3 and IGF-1, increased glucose transport is associated with reduced transporter K_m for glucose [23]. In another study with a large panel of malignant and non-malignant cell lines, whose growth in culture is dependent on components in fetal and newborn calf serum, malignancy was shown to be associated with a decrease in the K_m of hexose uptake [27]. In this study, it was suggested that reduced K_m for glucose may result from changes in glycosylation of a membrane protein associated with glucose transport. Although many studies support the concept of altered glycosylation of membrane proteins in malignant cells, including changes in glycosylation of the glucose transporter [48], our results suggest an alternative explanation in which altered phosphorylation of plasma membrane molecules associated with glucose transport may regulate glucose transport. Thus activation of phosphorylation cascades by oncogene-encoded tyrosine kinases may explain the reduced Michaelis constants associated with increased glucose uptake in malignancy. The possibility that such a change in the regulation of glucose transport may, at least in part, explain the changes in energy metabolism known for a long time to be associated with tumorigenesis [28] warrants further investigation.

This work was supported by the Health Research Council and by the Cancer Society of New Zealand. We wish to thank Dr. Bill Jordan for constructive comments, and Gabrielle Dennis for typing the manuscript.

REFERENCES

1 Carruthers, A. (1990) Physiol. Rev. **70**, 1135–1176
2 Cushman, S. W. and Wardzala, L. J. (1980) J. Biol. Chem. **255**, 4758–4762
3 Susuki, K. and Kono, T. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 2542–2545
4 Klip, A., Ramlal, T., Young, D. A. and Holloszy, J. O. (1987) FEBS Lett. **224**, 224–230
5 Hirshman, M. F., Goodyear, L. J., Wardzala, L. J., Horton, E. D. and Horton, E. S. (1990) J. Biol. Chem. **265**, 987–991
6 Whitesell, R. R. and Abumrad, N. A. (1985) J. Biol. Chem. **260**, 2894–2899
7 Toyoda, N., Flanagan, J. E. and Kono, T. (1987) J. Biol. Chem. **262**, 2737–2745
8 Suzuki, K. (1988) J. Biol. Chem. **263**, 12247–12252
9 Simpson, I. A. and Cushman, S. W. (1986) Annu. Rev. Biochem. **55**, 1059–1089
10 Gibbs, E. M., Allard, W. J. and Lienhard, G. E. (1986) J. Biol. Chem. **261**, 16597–16603
11 Allard, W. J., Gibbs, E. M., Witters, L. A. and Lienhard, G. E. (1987) Biochim. Biophys. Acta **929**, 288–295
12 Reusch, J. E., Begum, N., Sussman, K. E. and Draznin, B. (1991) Endocrinology **129**, 3269–3273
13 Begum, N., Leitner, W., Reusch, J. E., Sussman, K. E. and Draznin, B. (1993) J. Biol. Chem. **268**, 3352–3356
14 James, D. E., Hiken, J. and Lawrence, J. J. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 8368–8372
15 Lawrence, J. J., Hiken, J. F. and James, D. E. (1990) J. Biol. Chem. **265**, 19768–19776
16 Corvera, S., Jaspers, S. and Pasceri, M. (1991) J. Biol. Chem. **266**, 9271–9275
17 Reusch, J. E., Sussman, K. E. and Draznin, B. (1993) J. Biol. Chem. **268**, 3348–3351

18 Begum, N. and Draznin, B. (1992) J. Clin. Invest. **90**, 1254–1262
19 Zorzano, A., Wilkinson, W., Kotliar, N., Thoidis, G., Wadzinkski, B. E., Ruoho, A. E. and Pilch, P. F. (1989) J. Biol. Chem. **264**, 12358–12363
20 Holman, G. D., Kozka, I. J., Clark, A. E., Flower, C. J., Saltis, J., Habberfield, A. D., Simpson, I. A. and Cushman, S. W. (1990) J. Biol. Chem. **265**, 18172–18179
21 Harrison, S. A., Buxton, J. M. and Czech, M. P. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 7839–7843
22 Harrison, S. A., Buxton, J. M., Clancy, B. M. and Czech, M. P. (1991) J. Biol. Chem. **266**, 19438–19449
23 Nefesh, I., Bauskin, A. R., Alkalay, I., Golembo, M. and Ben-Neriah, Y. (1991) Int. Immunol. **3**, 827–831
24 Mueckler, M. and Lodish, H. F. (1986) Nature (London) **322**, 549–552
25 Whetton, A. D., Bazill, G. W. and Dexter, T. M. (1984) EMBO J. **3**, 409–413
26 Hamilton, J. A., Vairo, G. and Lingelbach, S. R. (1988) J. Cell. Physiol. **134**, 405–412
27 White, M. K., Bramwell, M. E. and Harris, H. (1983) J. Cell Sci. **62**, 49–80
28 Warburg, O. (1956) Science **123**, 309–314
29 Greenberger, J. S., Sakakeeny, M. A., Humphries, R. K., Eaves, C. J. and Eckner, R. J. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 2931–2935
30 Berridge, M. V., Tan, A. S. and Hilton, C. J. (1993) Exp. Hematol. **21**, 269–276
31 Esko, J. D., Gilmore, J. R. and Glaser, M. (1977) Biochemistry **16**, 1881–1890
32 Shetty, M., Loeb, J. N., Vikstrom, K. and Ismail-Beigi, F. (1993) J. Biol. Chem. **268**, 17225–17232
33 Salter, D. W. and Weber, M. J. (1979) J. Biol. Chem. **254**, 3554–3561
34 Hansen, M. B., Nielsen, S. E. and Berg, K. (1989) J. Immunol. Methods **119**, 203–210
35 Mosmann, T. (1983) J. Immunol. Methods **65**, 55–63
36 Berridge, M. V. and Tan, A. S. (1993) Arch. Biochem. Biophys. **303**, 474–482
37 Harrington, C. R. (1990) Anal. Biochem. **186**, 285–287
38 Kasanicki, M. A. and Pilch, P. F. (1990) Diabetes Care **13**, 219–227
39 Berridge, M. V., Horsfield, J. A. and Tan, A. S. (1994) J. Cell. Physiol., in the press
40 Bushart, G. B., Vetter, U. and Hartmann, W. (1993) Hormone Metab. Res. **25**, 210–213
41 Witters, L. A., Vater, C. A. and Lienhard, G. E. (1985) Nature (London) **315**, 777–778
42 Lawrence, J. J., Hiken, J. F. and James, D. E. (1990) J. Biol. Chem. **265**, 2324–2332
43 Piper, R. C., James, D. E., Slot, J. W., Puri, C. and Lawrence, J. C. (1993) J. Biol. Chem. **268**, 16557–16563
44 Bird, T. A., Davies, A., Baldwin, S. A. and Saklatvala, J. (1990) J. Biol. Chem. **265**, 13578–13583
45 Charron, M. J., Brosius, F. C., Alper, S. L. and Lodish, H. F. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 2535–2539
46 Haber, R. S., Weinstein, S. P., O'Boyle, E. and Morgello, S. (1993) Endocrinology **132**, 2538–2543
47 Yano, H., Seino, Y., Inagaki, N., Hinokio, Y., Yamamoto, T., Yasuda, K., Masuda, K., Someya, Y. and Imura, H. (1991) Biochem. Biophys. Res. Commun. **174**, 470–477
48 Bramwell, M. E., Davies, A. and Baldwin, S. A. (1990) Exp. Cell. Res. **188**, 97–104

Received 15 July 1994/25 August 1994; accepted 31 August 1994